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# Effect of removal of zinc on alfalfa mosaic virus RNA-dependent RNA polymerase

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The necessity of coat protein for infection of plants by alfalfa mosaic virus (AIMV) and other ilarviruses distinguishes this virus group from other plant virus groups. Recently, the presence of both a zinc-finger type motif and zinc in AIMV coat protein was described [(1989) *Virology* 168, 48–56]. We studied the effect of a zinc chelator on viral RNA synthesis. Strong inhibition of AIMV RNA-dependent RNA polymerase (RdRp) by ortho-phenanthroline (OP) was observed.

Alfalfa mosaic virus; Zinc; RNA polymerase inhibition

## 1. INTRODUCTION

Alfalfa mosaic virus (AIMV) requires a few copies of its coat protein bound to the genomic RNAs to initiate infection in host plants. This phenomenon called genome activation distinguishes AIMV and other ilarviruses from all other plant viruses. Coat protein binds preferentially to the homologous 3'-terminal regions of genomic RNAs (for review see [1]). It has been postulated that the coat protein is involved in viral RNA synthesis (for review see [1]). The isolation of an RNA-dependent RNA polymerase (RdRp) which is capable of initiation *de novo* on added templates [2] provides a tool to elucidate the role of the coat protein in RNA synthesis. The recent report on the presence of both a putative 'zinc-finger' type domain and zinc itself in the coat proteins of the ilarviruses AIMV and tobacco streak virus [3] and the rapidly accumulating data on the importance of 'zinc-finger' domain-containing proteins in eukaryotic transcription regulation [4] prompted us to investigate the effect of removal and addition of zinc on AIMV RdRp activity *in vitro*.

## 2. METHODS AND MATERIALS

AIMV RdRp was prepared as described previously [2]. RNA synthesis by this enzyme preparation is fully dependent on added templates, even when micrococcal nuclease treatment was omitted (data not shown).

To 5  $\mu$ l of the enzyme preparation  $ZnCl_2$ , ortho-phenanthroline (OP) or combinations of both were added as indicated in the legends of the figures. The volume was adjusted to 50  $\mu$ l with buffer (final concentrations: 50 mM Tris-HCl, pH 8.2; 10 mM  $MgCl_2$ ; 10 mM

dithiothreitol (DTT); 15% (v/v) glycerol). Enzyme preparations were incubated with zinc-ions and chelator for 20 min at 25°C. Subsequently, 50  $\mu$ l reaction buffer (50 mM Tris-HCl, pH 8.2; 10 mM  $MgCl_2$ ; 10 mM DTT; 2 mM ATP, CTP, GTP; 20  $\mu$ M UTP; 10  $\mu$ Ci [ $\alpha$ - $^{32}$ P]UTP, 400 Ci/mmol; 400  $\mu$ g/ml template RNAs) was added. Reactions were carried out at 30°C for 30 min.

Reaction products were processed, treated with nuclease S1 and analyzed by gel electrophoresis as described previously [4].

## 3. RESULTS AND DISCUSSION

Ortho-phenanthroline is a well-defined chelator of zinc and proved to be effective in systems in which other metal chelators were ineffective [5]. In addition to OP we tested the chelators ethyleneglycolbis( $\beta$ -amino-ethyl ether)*N,N'*-tetraacetic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA) for their capability to inhibit AIMV RdRp activity. No significant inhibition of AIMV RdRp activity by EGTA and EDTA at a concentration of 5 mM was observed (data not shown). High concentrations (20 mM) of EDTA did inhibit RdRp activity probably by removal of  $Mg^{2+}$  ions essential for polymerase activity. OP has a strong inhibitory effect on AIMV RdRp activity (Fig. 1, lane 2; Fig. 2). OP at a concentration of 2 mM inhibits AIMV RdRp activation for more than 50% while complete inhibition of RdRp activity is observed at concentrations 6 mM and higher (Fig. 2). The inhibition is not observed if zinc ions are added simultaneously with the chelator (Fig. 1, lane 3) or just prior to incubation with AIMV RNAs and nucleosidetriphosphates (Fig. 1, lane 5). This indicates that the inhibition is caused by the removal of zinc from the RdRp preparation and is not due to impurities in the OP preparation. AIMV RdRp is slightly stimulated by the addition of zinc ions (Fig. 1, lane 5). Addition of divalent metal ions:  $Ca^{2+}$ ,  $Co^{2+}$ ,  $Cu^{2+}$ ,  $Mg^{2+}$  and  $Mn^{2+}$  did not restore the activity of

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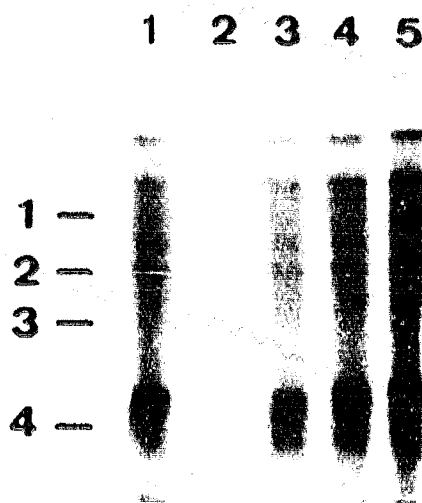


Fig. 1. Autoradiogram of double-stranded products synthesized in reaction mixtures containing: untreated AIMV RdRp (lane 1); AIMV RdRp preincubated with 10 mM OP (lane 2); AIMV RdRp preincubated with 10 mM OP and 10 mM  $ZnCl_2$  added simultaneously prior to RNA synthesis (lane 3); AIMV RdRp preincubated with 10 mM OP, 10 mM  $ZnCl_2$  was added just prior to RNA synthesis (lane 4); AIMV RdRp preincubated with 10 mM  $ZnCl_2$  (lane 5). Positions of AIMV virion RNAs are indicated.

OP-treated AIMV RdRp or stimulated AIMV RdRp activity (data not shown). Since DTT is a weak chelator of zinc the experiments were repeated using  $\beta$ -mercaptoethanol as a reducing agent. However, in none of the experiments a difference was observed using this agent instead of DTT (data not shown).

From the data presented in this paper it is clear that a zinc-dependent factor is involved in AIMV RNA synthesis. Since no double-stranded reaction products of any size were detected after removal of zinc from AIMV RdRp either an early event in viral RNA synthesis e.g. initiation is inhibited or the structural integrity is reversibly disrupted by zinc removal. It is tempting to conclude that removal of zinc from the AIMV coat protein is the cause of the inhibition of RdRp activity. However, it is equally well possible that removal of zinc from another protein in the crude enzyme preparation is responsible for the inhibition of RdRp activity. Further purification and characterization of the AIMV RdRp should provide more information on the nature

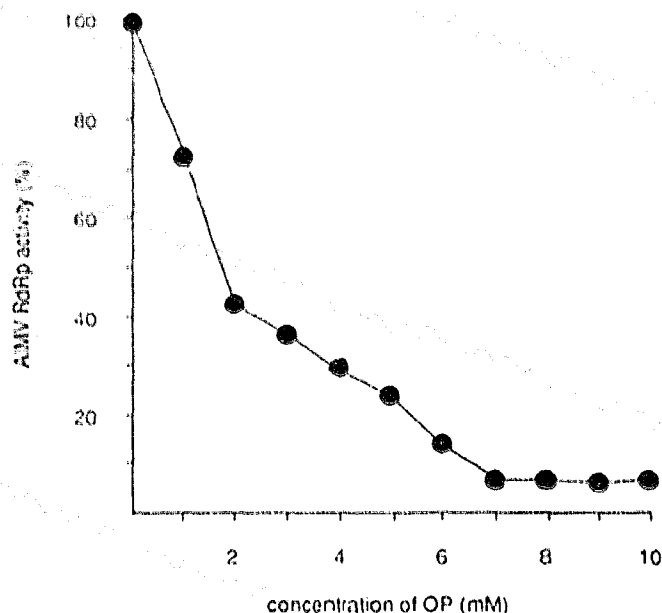


Fig. 2. Inhibition of AIMV RdRp by OP. AIMV RdRp was preincubated with OP at concentrations indicated in the graph. Incorporation of  $[^{32}P]$ UMP in double-stranded products synthesized by OP-treated AIMV RdRp was determined. Incorporation of  $[^{32}P]$ UMP by non-treated AIMV (11 547 cpm) was set to 100% activity.

of the zinc-dependent factor present in the AIMV RdRp complex.

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